

State of Hawaii  
DEPARTMENT OF LAND AND NATURAL RESOURCES  
Division of Aquatic Resources  
Honolulu, Hawaii 96813

April 28, 2006

Board of Land  
and Natural Resources  
Honolulu, Hawaii

THE DIVISION OF AQUATIC RESOURCES REQUESTS BOARD OF LAND AND  
NATURAL RESOURCES (BLNR) AUTHORIZATION/APPROVAL TO ISSUE ONE  
(1) NORTHWESTERN HAWAIIAN ISLANDS (NWHI) RESEARCH, MONITORING  
AND EDUCATION PERMIT TO DR. ROBERT TOONEN OF THE HAWAII  
INSTITUTE OF MARINE BIOLOGY, FOR THE TAKE OF INVERTEBRATES FOR  
GENETIC SAMPLING IN ORDER TO STUDY CORAL REEF CONNECTIVITY

Submitted herewith for your authorization and approval is a request for issuance of a NWHI Access Permit to Dr. Robert Toonen of the Hawaii Institute of Marine Biology (HIMB), University of Hawaii. The Research, Monitoring and Education Permit, described below, will allow activity to occur in the NWHI State marine Refuge (0-3 miles) waters surrounding Nihoa Island, French Frigate Shoals, and Gardner Pinnacles. The activities covered under this permit will occur from May 18 to June 11, 2006, from the support vessel Hi'ialakai. Ship details are provided with Item F-4.

Toonen will be conducting a genetic survey of invertebrates, intended to address the issue of population connectivity across the NWHI. He proposes to survey 29 species/taxa across the entire archipelago, using mtDNA sequencing technologies and genotyping of nuclear (microsatellite) markers. The motivation for these studies is to resolve larval dispersal between reef habitats, to bolster the scientific foundations for conservation of the Northwest Hawaiian Islands. In particular, this research will provide an evaluation of the fundamental connections (or lack thereof) between isolated reef habitats in the NWHI. The level of isolation of invertebrate populations within the Refuge is very important information for management purposes. This information can tell us, e.g., whether there is one stock of a particular invertebrate species, or several, rarer, sub-stocks (or even "cryptic species" - new species of invertebrate that are morphologically similar/identical to existing species). Obviously, management strategies will change dependent on whether invertebrate populations of the NWHI are found to be isolated management units, or a single interactive metapopulation.

The proposed activities (below) are consistent with and support the purposes of the Refuge, primarily to better understand and manage the resources within the marine refuge.

Toonen and sub-permittees propose to take samples of invertebrates from Nihoa, FFS, and Gardner Pinnacles (see attached species list). Greater than 93% of his samples are

non-lethal (marked with an asterisk in the species table), involving biopsy of a small, rice-grain sized tissue sample. Samples of corals are to be shared with other HIMB personnel submitting separate permit applications (see Items F-8, F-9). Samples will be analyzed genetically at the Hawaii Institute of Marine Biology after the research cruise, as detailed in the Permit Application.

#### REVIEW PROCESS:

This permit application was received by the Division of Aquatic Resources on March 9, 2006. It was sent out for review and comment to the following scientific entities: Division of Aquatic Resources staff (5), Division of Forestry and Wildlife, Northwest Hawaiian Islands Reserve, and the United States Fish and Wildlife Service. Native Hawaiians from the Office of Hawaiian Affairs, and Kaho'olawe Island Reserve Commission were also consulted.

Comments received from the Scientific Community (DAR and the NWHI Reserve) are summarized as follows:

- 1) Concern was expressed over collections of uncommon species such as banded coral shrimp, white spotted hermit crab, solitary and cup corals, and spiny and slipper lobsters
- 2) Concern was expressed that Toonen asked for larger sample sizes than Bowen, who is using similar molecular techniques to analyze fish populations

Comments received from a Native Hawaiian are summarized as follows:

1. There was concern for native Hawaiian intellectual property rights for new discoveries and the protection of the resources for their potential product developments.

#### RESPONSE:

A meeting of DAR staff and HIMB researchers was held on 12 April 2006 to address concerns, and a synopsis of the response to concerns raised is as follows:

- 1) It was discovered in the meeting that an error had been made in the permit submission, and asterices indicating non-lethal sampling were omitted. All the species of concern were to be non-lethally sampled. Toonen provided an amended version of the permit application clarifying this. He has found 100% survival in previous studies taking a rice-grain sized biopsy of hermit crabs, corals and other invertebrates. This information allayed the concerns of reviewers.
- 2) Toonen explained that invertebrates have larger genetic effective population sizes with greater diversity, meaning that in order to detect differences Toonen needs larger samples than does Bowen.
- 3) The Guidelines for Submitting Permit Applications stipulates that, for all permits, the activity must be non-commercial and will not involve the sale of any organism, byproduct, or material collected. Furthermore, the Guidelines state that

resources and samples are a public trust, and are not to be used for sale, patent, bioassay, or bio-prospecting, or for obtaining patents or intellectual property rights. This condition will be added to the Permit Terms and Conditions for this, and all future permits. This should address the concerns raised by the Native Hawaiian reviewer.

AMENDMENTS REQUESTED SUBSEQUENT TO APPLICATION SUBMISSION:

Toonen requested (in writing) the inclusion of the following personnel as sub-permittees, in addition to those originally stipulated: Scott Godwin, Erik Franklin, Jill Zamzow, Randy Kosaki. He also requested the addition of two species to his table (present in his revised application), *Pavona varians* (biopsies) and assorted Trapezid crabs (whole organism, for the Bishop Museum's collection).

FINAL STAFF RECOMMENDATIONS:

- 1) Approve the take of non-lethal and lethal invertebrate samples of the species, numbers, and locations indicated in Toonen's revised sampling table (attached), with the conditions stipulated above.
- 2) Approve the addition of the requested sub-permittees
- 3) Coral samples are to be taken in coordination with other HIMB personnel: the stated sample sizes and numbers are to be shared by Toonen, Aeby, Karl, Rappé and Gates (see separate permit applications).

RECOMMENDATION:

"That the Board authorize and approve, with stated conditions, a Research, Monitoring and Education Permit to Dr. Robert Toonen of the Hawaii Institute of Marine Biology, for activities and access within the State waters of the NWHI."

Respectfully submitted,



DAN POLHEMUS  
Administrator

APPROVED FOR SUBMITTAL



PETER T. YOUNG  
Chairperson

## APPENDIX 1

**State of Hawai'i  
DLNR  
Northwestern Hawaiian Islands State Marine  
Refuge  
Permit Application Form**

<i>For Office Use Only</i>
Permit No:
Expiration date:
Date Appl. Received: <u>3/8/06</u>
Appl. Fee received: <u>N/A</u>
NWHI Permit Review Committee date:
Board Hearing date:
Post to web date:

### Type of Permit

- ☒ I am applying for a **Research, Monitoring & Education** permit. (Complete and mail Application)
- ☒ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.
- ☐ I am applying for a permit for a **Native Hawaiian** permit. (Complete and mail Application)
- ☐ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.
- ☐ I am applying for a **Special Activity** permit. (Complete and mail Application)
- ☐ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

Briefly describe **Special permit** activity:

When will the NWHI activity take place?

- ☒ **Summer** (May-July of 2006 (year)

Note: Permit request must be received before February 1st

Specific dates of expedition May 18 - June 14, 2006

- ☒ **Fall** (August-November) of 2006 (year)

Note: Permit request must be received before May 1<sup>st</sup>

Specific dates of expedition TBA

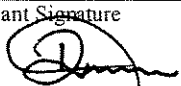
- ☐ **Other**

**NOTE: INCOMPLETE APPLICATIONS WILL NOT BE ACCEPTED**

**Please Send Permit Applications to:**

NWHI State Marine Refuge Permit Coordinator  
State of Hawai'i  
Department of Land and Natural Resources  
Division of Aquatic Resources  
1151 Punchbowl Street, Room 330  
Honolulu, Hawai'i 96813

**NWHI State Marine Refuge Permit Application**  
**See Appendix 2 for Application Instructions**

Section A – Applicant Information	
1. Project Leader (attach Project Leader's CV or resume) <input checked="" type="checkbox"/> CV attached <p style="text-align: center;">Toonen, Robert</p>	<p style="text-align: center;">Assistant researcher</p>
Name: Last, First, Middle Initial 2. Mailing Address (Street/PO Box, City, State, Zip) <p style="text-align: center;">Hawaii Institute of Marine Biology            P.O. Box 1346            Kaneohe HI 96744</p>	Title Telephone ( 808 ) 236-7425 Fax ( 808 ) 236-7443 Email Address toonen@hawaii.edu
3. Affiliation (Institution/Agency/Organization) <p style="text-align: center;">University of Hawaii</p>	For graduate students, Major Professor 's Name & Telephone
4. Sub-Permittee/Assistant Names, Affiliations, and Contact Information <input type="checkbox"/> CV or resume attached <p style="text-align: center;">Iliana Baums (Ph.D.), Steven Karl (Ph.D.), CV's attached</p>	
5. Project Title <p style="text-align: center;">HIMB-NWHI Coral Reef Research Partnership: Connectivity of coral reef fishes</p>	
6. Applicant Signature 	7. Date (mm/dd/yyyy) <p style="text-align: center;">03/07/2006</p>

Section B: Project Information
8. (a) Project Location <input checked="" type="checkbox"/> NWHI State Marine Refuge (0-3 miles) waters surrounding: <div style="margin-left: 20px;"> <input checked="" type="checkbox"/> Nihoa Island  <input type="checkbox"/> Necker Island (Mokumanamana)  <input checked="" type="checkbox"/> French Frigate Shoals  <input type="checkbox"/> Laysan  <input type="checkbox"/> Maro  <input checked="" type="checkbox"/> Gardner Pinnacles  <input type="checkbox"/> Lisianski Island, Neva Shoal  <input type="checkbox"/> Pearl and Hermes Atoll  <input type="checkbox"/> Kure Atoll, State Wildlife Refuge  <input type="checkbox"/> Other NWHI location         </div> <p>Describe project location (include names, GPS coordinates, habitats, depths and attach maps, etc. as appropriate).</p> <p style="margin-top: 20px;">see attached documentation.</p>

**(b) check all actions to be authorized:**

- ☒ Enter the NWHI Marine Refuge waters
- ☒ Take (harvest)      ☒ Possess      ☒ Transport (☒ Inter-island    ☐ Out-of-state)
- ☒ Catch      ☒ Kill      ☐ Disturb    ☐ Observe
- ☐ Anchor      ☐ Land (go ashore)      ☐ Archaeological research
- ☐ Interactions with Sea Turtles or Monk Seals    ☐ Interactions with Seabirds
- ☒ Interactions with Live Coral, Ark Shells or Pearl Oysters
- ☐ Interactions with Jacks, Grouper or Sharks
- ☐ Conduct Native Hawaiian religious and/or cultural activities
- ☐ Other activities \_\_\_\_\_

**(c) Collection of specimens – collecting activities (would apply to any activity):**

**Organisms or objects (List of species, if applicable, add additional sheets if necessary):**

Common name	Scientific name	No. & size of specimens	Collection Location(s)
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See attached

**(d) What will be done with the specimens after the project has ended?**

Specimens will be archived in 70% Ethanol, specimen data will be recorded in an Access database, DNA will be stored in -80 freezer.

**(e) Will the organisms be kept alive after collection?**    ☐ yes    ☒ no

- Specific site/location \_\_\_\_\_
- Is it an open or closed system?    ☐ open    ☐ closed
- Is there an outfall?    ☐ yes    ☐ no
- Will these organisms be housed with other organisms? If so, what are the other organisms?

(Please attach additional documentation as needed to complete the questions listed below)

<p>9. Purpose/Need/Scope:</p> <ul style="list-style-type: none"> <li>This research is a genetic survey of reef invertebrates, designed to address the issue of population connectivity across the NWHI. We will survey approximately 30 species across the entire archipelago, using mtDNA sequencing and genotyping technology. See attached.</li> </ul>
<p>Describe how your proposed activities will help provide information or resources to fulfill the State Marine Refuge purpose and to reach the Refuge goals and objectives.</p> <ul style="list-style-type: none"> <li>The motivation for these population connectivity studies is to resolve larval dispersal between reef habitats, to bolster the scientific foundations for conservation of the Northwest Hawaiian Islands. See attached documentation.</li> </ul>
<ul style="list-style-type: none"> <li>Describe context of this activity, include history of the science for these questions and background.</li> </ul> <p>The fundamental units of wildlife management are isolated stocks and ecosystems. The research will use advanced molecular technologies to resolve these units in the NWHI.</p>
<ul style="list-style-type: none"> <li>Explain the need for this activity and how it will help to enhance survival or recovery of refuge wildlife and habitats.</li> </ul> <p>See attached documentation.</p>
<ul style="list-style-type: none"> <li>Describe how your proposed project can help to better manage the State Marine Refuge.</li> </ul> <p>See attached documentation.</p>
<p>10. Procedures (include equipment/materials)</p> <p>See attached documentation.</p>
<p>11. Funding sources (attach copies budget &amp; funding sources).</p> <p>See attached documentation.</p>
<p>12. List all literature cited in this application as well as all other publications relevant to the proposed project.</p> <p>See attached documentation.</p>
<p>13. What types of insurance do you have in place? (attach documentation)</p> <p><input checked="" type="checkbox"/> Wreck Removal</p> <p><input checked="" type="checkbox"/> Pollution</p>
<p>14. What certifications/inspections do you have scheduled for your vessel? (attach documentation)</p> <p><input checked="" type="checkbox"/> Rat free      <input type="checkbox"/> tender vessel      <input type="checkbox"/> gear/equipment</p> <p><input checked="" type="checkbox"/> Hull inspection      <input checked="" type="checkbox"/> ballast water</p>
<p>15. Other permits (list and attach documentation of all other required Federal or State permits).</p> <p>Permits from the U.S.F.W.S. and the NWHI Coral Reef Ecosystem Reserve are pending</p>
<p>16. Project's relationship to other research projects within the NWHI State Marine Refuge, National Wildlife Refuge, NWHI Coral Reef Ecosystem Reserve, or elsewhere.</p> <p>See attached documentation.</p>

### Section C: Logistics

**17. Time Frame:**

Project Start Date

05/01/2005

Project Completion Date

12/31/2009

Dates actively inside the State Marine Refuge.

05/19/2006 - 05/31/2006

Personnel schedule in the State Marine Refuge (describe who will be where and when).

See attached documentation.

**18. Gear and Materials**

☒ Dive equipment

☐ Radio Isotopes

☒ Collecting Equipment

☐ Chemicals (specify types)

**19. Fixed installations and instrumentation.**

☐ Transect markers

☐ Acoustic receivers

☐ Other (specify)

**20. Provide a time line for sample analysis, data analysis, write-up and publication of information.**

See attached documentation.

**21. Vessel Information:**

Vessel Name Hi'ialakai

IMO Number 8835619

Vessel Owner NOAA

Flag USA

Captain's Name CDR S. Kuester

Chief Scientist or Project Leader R. Kosaki

Vessel Type R/V

Call sign WTEY

Length 224 ft

Gross tonnage 1,914

Port of Embarkation Honolulu, HI

Last port vessel will have been at prior to this embarkation Am Sam

Total Ballast Water Capacity: Volume see attached m<sup>3</sup> Total number of tanks on ship 10

Total Fuel Capacity: 228,642 Total number of fuel tanks on ship 15

Other fuel/chemicals to be carried on board and amounts:

See attached documentation.

Number of tenders/skiffs aboard and specific type of motors:

See attached documentation.

Does the vessel have the capability to hold sewage and grey-water? Describe in detail.

See attached documentation.

Does the vessel have a night-time light protocol for use in the NWHI? Describe in detail (attach additional pages as necessary)

See attached documentation.

On what workboats (tenders) will personnel, gear and materials be transported within the State Marine Refuge?

See attached documentation.

How will personnel, gear and materials be transported between ship and shore?

See attached documentation.

If applicable, how will personnel be transported between islands within any one atoll?

See attached documentation.



## Northwestern Hawaiian Islands State Marine Refuge

### Permit Application Form

Supplemental Information

Toonen, R.

*Associated personnel:*

Iliana Baums, Scott Godwin, Erik Franklin, Jill Zamzow, Randy Kosaki.

#### 8a. Project Location

Nihoa	23° 03' 39" North	161° 56' 07" West
French Frigate Shoals	23° 43' 51" North	166° 09' 54" West
Gardner Pinnacles	25° 21' 58" North	170° 31' 09" West

Our request is to operate from dive boats launched from the NOAA vessel Hi'ialakai, collecting specimens in 10-100 feet depth on coral reef habitat. Collections will be spread across several adjacent reefs in order to minimize impact on ecosystem function.

#### 8c. Collection of Specimen

To assess population connectivity, we need samples of up to 50 specimens per location, from the three locations described above, for a total of up to 150 specimens per species. Of course actual sample sizes will be lower because not all sampling goals will be met at all locations, and at some locations (French Frigate Shoals) previous collections need only be supplemented to the total requested sample size. The target size for genotyping is 50 samples, and the target size for DNA sequencing is 30 samples. Samples which are collected by non-lethal biopsy are marked with an asterisk (\*). Of each species, specimens of all sizes will be collected. Nihoa (NI), French Frigate Shoals (FR) and Gardener Pinnacles (GA). Coral samples will be shared with Steve Karl, Ruth Gates & Mike Rappe (see Karl, Gates & Rappe permit applications).

Groups	Subgroups	Species	N	Sites
Cnidaria	Hard corals	<i>Porites lobata</i> *	50	NI
			50	GA
			20	FR^
		<i>Montipora capitata</i> *	50	NI
			50	GA
			50	FR^
		<i>Acropora cytherea</i> *	50	NI
			50	GA
			30	FR
		<i>Fungia scutaria</i> *	50	NI
			50	GA
			46	FR
		<i>Pocillopora menadrina</i> *	50	NI
			50	GA

Groups	Subgroups	Species	N	Sites
Crustacea		<i>Pocillopora damicornis</i> *	50	FR
			50	NI
			50	GA
			50	FR
		<i>Pavona varians</i> *	50	NI
			50	GA
			30	FR
		<i>Tubastraea coccinea</i> *	50	NI
			50	GA
			50	FR
	Soft corals	Invasive <i>Carijoa</i> if encountered <sup>†</sup>	x	NI
			x	GA
			x	FR
	Hermit crabs	<i>Calcinus hazletti</i>	30	NI
			30	GA
			10	FR
		<i>Calcinus laevimanus</i>	30	NI
			30	GA
			30	FR
		<i>Dardanus megistos</i> *	30	NI
			30	GA
			30	FR
	Coral banded shrimp	<i>Stenopus hispidus</i> *	30	NI
			30	GA
			29	FR
	Trapezid crabs	Species collection for Bishop Museum (not to exceed 50 individuals/site)	50	NI
			50	GA
			50	FR
	Spiny & Slipper lobster	<i>Panulirus marginatus</i> *	30	NI
			30	GA
			30	FR
		<i>Scyllarides squammosus</i> *	30	NI
			30	GA
			30	FR
Echinodermata	Sea cucumbers	<i>Holothuria atra</i> *	50	NI
			50	GA
			47	FR
		<i>Holothuria whitmaei</i> *	50	NI
			50	GA
			36	FR
	Brittle star	<i>Ophiocoma erinaceus</i> *	50	NI
			50	GA
			22	FR
		<i>Ophiocoma pica</i> *	50	NI
			50	GA
	Sea stars	<i>Linckia multifora</i> *	50	FR
			50	NI
			50	GA
			24	FR

Groups	Subgroups	Species	N	Sites
		<i>Acanthaster planci</i> *	30	NI
			30	GA
			30	FR
		<i>Heterocentrotus mamillatus</i> *	50	NI
			50	GA
			49	FR
		<i>Eucidaris metularia</i> *	50	NI
			50	GA
			44	FR
	Shingle urchin	<i>Colobocentrotus mertensi</i> *	50	NI
			50	GA
			50	FR
	Reef urchins	<i>Tripneustes gratilla</i> *	30	NI
			30	GA
			30	FR
Mollusca	Opihi	<i>Cellana</i> spp.	10	NI
			10	GA
			10	FR
	Octopus	<i>Octopus</i> spp.*	10	NI
			10	GA
			10	FR
Bryozoa	Moss animal	<i>Reteporellina dentriculata</i> *	50	NI
			50	GA
			50	FR

\* *Carijoa riisei* has not been reported in the NWHI to date, but if it is found, we plan to sample any colonies located.

## 9. Purpose/ Need/ Scope

This research is a genetic survey of reef corals and other reef invertebrates, designed to address the issue of population connectivity across the central and eastern Pacific with special focus on their importance as propagule donors to the North Western Hawaiian Islands. We will survey approximately 27 invertebrate species across the entire range, using mtDNA sequencing and microsatellite genotyping technology. With a few notable exceptions (e.g. Rivera et al. 2004; Baums et al. 2005b) reef corals and other reef invertebrates have not been surveyed on this scale. Although the State prohibits extraction of any coral or live rock from State waters, laws cannot prevent natural and human-made disasters such as hurricanes, storms, warm/cold-water events, coral bleaching, marine debris, ship groundings, oil spills, disease outbreaks, etc. It is of critical importance to state managers to know how our precious marine resources will respond to these impacts and how such impacts can be mitigated. On one end of the extreme, the individual locations within the State Refuge are isolated populations with reduced genetic diversity, increased risk of extinction, and little hope of recovery from such local disasters within the lifetime of a manager. On the other end, the reefs are fully integrated populations which will stand or fall as an entire chain, and the local effects at any given location should have no long-term ramifications at any other location. This research seeks to provide an answer on where along this continuum individual locations within the State Refuge lies.

Describe how your proposed activities will help provide information or resources to fulfill the State Marine Refuge purpose and to reach the Refuge goals and objectives.

The proposed research is a multispecies approach to assess population connectivity and evolutionary partitions among tropical reef habitats of the Hawaiian archipelago and their connection to the central and eastern Pacific. The immediate motivation for this work is to bolster the scientific foundations for implementing the NWHI National Marine Sanctuary.

However understanding the patterns and consequences of connectivity has direct management implications for the State Refuge as well. This research will provide a better understanding of the degree to which populations are demographically open, such that depleted populations can be replenished by recruitment from distant healthy populations, or demographically closed, and thus in need of local management. Further use for these data involves gaining an understanding of the standing genetic diversity by location throughout the State Refuge. Several recent studies have shown a direct link between demographic population structure and genetic population structure (Selkoe, in review; Toonen & Grosberg in review). Further, Frankham and colleagues have recently shown that across 170 of the most threatened species on the planet, 77% of them had significantly lower genetic diversity than that found in the closest-related nonthreatened species (Spielman, Brook & Frankham, 2004; Frankham, 2005). Both subsequent empirical and theoretical work suggests that reductions in genetic diversity and the resultant inbreeding can speed the mean time to extinction by up to 78% over species without reduced genetic diversity. These genetic data surveys have obvious and immediate management implications; this survey should highlight some locations and species most likely to be at risk of extinction within the State's Refuge.

Finally, we are seeking to cover a broad range of taxonomic and habitat types to gain some inference about whether the patterns and consequences of connectivity can be generalized by life history, taxonomy or habitat, or whether each species is a unique case that requires individual study to draw any management implications. This question can only be addressed by doing a reasonable survey of common species across each of these factors for statistical comparison. The management implications of such efforts are obvious: if generalizations can be drawn among any such groups, then even in the absence of specific data, a guided management response can be launched quickly as the need arises. In contrast, if no such generalizations can be made, and the results must be determined on a case-by-case basis, then in the event of an unforeseen management requirement, the absence of data must result in an uninformed response on the part of State managers.

Describe context of this activity, include history of the science for these questions and background.

Reef corals and other reef invertebrates typically have a pelagic phase (eggs and larvae), which lasts 20-60 days, followed by settlement onto a reef where they remain through juvenile and adults stages. Long distance dispersal is accomplished almost exclusively during the pelagic larval phase. However, the geographic limits of such dispersal are uncertain. Recent research shows that effective dispersal of marine larvae can fall far short of their potential (reviewed by Swearer et al. 2002).

These findings set the stage for a methodical range-wide survey of reef faunas in the eastern and central Pacific. Advances in population genetics, especially Bayesian assignment tests for microsatellite markers and coalescence theory for sequencing data, will greatly enhance our analysis, elucidating the direction and scale of gene flow as well as the history of populations (reviewed by Emerson et al. 2001; Arbogast et al. 2003; Manel et al. 2005).

Mt DNA approaches have proven unsuccessful for reef corals (Shearer et al. 2002) so that we will use microsatellite markers to elucidate patterns of gene flow and describe population structure of these important ecosystem engineers (Baums et al. 2005a; Baums et al. 2005b).

The timing and pathway of Hawaiian colonization events is also of interest. Gosline (1955) proposed that at low sea level stands associated with glaciation, the Northern Equatorial Current (and countercurrent) may have been deflected by the Line Islands, providing greater opportunities for colonization into Hawaii. Although phylogeographic approaches have become the dominant approach to test such hypotheses in other systems (reviewed by Avise 2000), they have not yet been applied to understand historical patterns of colonization of the Hawaiian Archipelago by marine organisms.

A genealogical approach to relationships among mtDNA haplotypes, including statistical parsimony (Templeton et al. 1992; Clement et al. 2000) will indicate whether the closest relatives to the Hawaiian fauna lie predominantly to the West (Ogasawara Arch, Wake Island, or Marshall Islands) or to the South (Line Islands, see Maragos and Jokiel 1986; Maragos et al. 2004). In these cases, populations of the widespread Indo-Pacific species will be compared to the Hawaiian endemic (sister taxon; Table 1). The geographic source of the Hawaiian form will be resolved with parsimony networks and phylogenetic tools (see Methods), and the age of colonization events will be estimated with the mtDNA molecular clock.

Explain the need for this activity and how it will help to enhance survival or recovery of refuge wildlife and habitats.

As outlined previously, marine reserves are the proposed tool for conservation and management of coral reef organisms in the North Western Hawaiian Islands. Optimal design strategies, i.e. their placement, number and size, are the subject of much debate (Palumbi, 2003). The number of separate populations and evolution units of reef organisms throughout the archipelago and the direction of gene flow among these units will determine how effective they may be as a source of larvae for neighboring imperiled reefs.

Describe how your proposed project can help to better manage the State Marine Refuge.

These data will provide direct information on connectivity required for management, and can also detect cryptic endemic species and document the patterns and history of species entering the Hawaiian Archipelago. Furthermore, by documenting the pattern and magnitude of connectivity in a diverse set of invertebrate taxa, we can determine if there are general patterns that can guide management decisions for understudied species. The

genetic surveys of connectivity among reef habitats will substantially augment the scientific foundation for conservation measures. Specifically, this research will establish whether reef ecosystems of the NWHI are isolated management units (as some preliminary data indicate) or components of an interactive metapopulation (as other preliminary data indicate). In the former case, each reef ecosystem will have to recover from environmental insults (whether human or natural) without significant input from other reef ecosystems. A corresponding conservation mandate would be that each ecosystem is an independent management unit. An ongoing debate about the NWHI is whether this is a series of relatively fragile (isolated) ecosystems, or whether it is a single robust ecosystem that can sustain extraction of resources. There is also a direct management concern about whether the NWHI serves as a source or a sink for the main Hawaiian Islands. The assays of population connectivity outlined here will go a long way towards settling these issues in a format that has statistical power and scientific credibility.

## **10. Procedure**

The objective of this research is to survey 27 key reef species across the central and eastern Pacific to assess the level of connectivity among isolated reef habitats. These species are described in Appendix 1. We will accomplish this with samples up to 50 invertebrate specimens/species/location, from taxa distributed across the region (Appendix 1). The “key” species in this case are locally abundant and widespread in the area, easy to identify, and easy to collect. Although some species may be listed as rare in the broad-scale NOWRAMP surveys, these species can still be very abundant in some specific locations, and we are only interested in sampling these locations discovered during previous cruises to the NWHI. If we are unable to collect 30-50 samples of these species, we are not interested in them because we do not have the statistical rigor to complete the proposed study. Also, our collection criterion is that we do not sample individuals if our collection would impact more than 1% of the estimated standing population at any atoll.

### Techniques

The target species (see 8c) inhabit shallow reefs and are accessible via snorkeling, or scuba dives. Tissue biopsy samples are typically obtained as small fragments for corals (1cm<sup>2</sup>), arm clips for sea stars, brittle stars and sea cucumbers, and whole animals for smaller invertebrates. Despite the fact that we target only extremely abundant species, for which our sampling would have no detectable population effects, whenever possible we obtain non-lethal tissue samples to further minimize our impact. Photographic monitoring of coral tissue sampling using the same methods has proposed here has shown that the small damage inflicted is fully repaired within approx. 2 months and no negative impact on coral colony health was apparent (Photographs available upon request). Coral reef invertebrates are collected by hand using pliers, forceps or hammer and chisel depending on the species. We store tissue samples in 70% Ethanol at room temperature in 2 ml vials during fieldwork. Samples are archived in a database and prepared for long-term storage and future DNA use upon return from the field.

The tissue biopsy collections are typically 0.1 – 0.5 grams (roughly the size of a grain of rice) and do not kill the animals that are sampled. Whenever possible, individuals are

sampled in a non-lethal manner (i.e., we take a tiny tissue sample from the animal and release it alive in the same location). For example, lobster and some crab samples involve only the excision of a single dactyl (toe) from the animal before it is released. By comparison, the normal anti-predatory defense response of these species is autotomy, which involves the ready self-amputation of an entire walking leg. In studies with porcelain crabs, and spiny lobsters in California, we showed a 100% survival rate among individuals kept in the lab for 2 months after being sampled in this way (Toonen, unpubl. data). Thus, we have no reason to expect this sampling effort would have any detectable negative impact on the populations sampled. Exceptions to non-lethal sampling include only hermit crabs of the genus *Calcinus*, in which we: 1) have preliminary data to expect that there may be some cryptic species, and we require the original specimens to sort out the taxonomy of the sampled animals after genetic analyses and 2) which are common by any measure of abundance across the NWHI archipelago. Such cases are of direct influence on the management decisions of the State, because we may discover previously unknown endemic species that are only identified after genetic typing.

Any sample size greater than  $N = 1$  can be informative in genetic analysis, but statistical rigor requires  $N > 25$  and our target sample size is 30-50/location (depending on the genetic marker being used – see Analysis below) for the target species. Target species are described in the table presented in 8c.

We plan to collect between 30 and 50 specimens/species for invertebrates around Nihoa, Gardner Pinnacles and French Frigate Shoals. However, we expect to fall far short of those numbers for most species on this collecting trip. For French Frigate Shoals, our target sample sizes are often smaller because we have obtained some samples from this location on a previous trip (September 2006). In addition, coral samples will be shared with other researchers partaking in the cruise (Dr. Stephen Karl, Dr. Mike Rappe and Dr. Ruth Gates). Our goal will be to focus on the species that can be collected rapidly, safely and in sufficient number for statistical rigor, and those priorities will be dictated in part by field conditions.

### Analysis

Two lab methodologies will be employed in this study. One will be direct sequencing of mitochondrial (mtDNA) genes using PCR methodology. In most species, a segment of approximately 800 base pairs of the mtDNA cytochrome *b* or cytochrome oxidase subunit I gene will be amplified and sequenced following protocols used daily in our laboratory. DNA sequences will be generated with an ABI 3100 automated DNA sequencer in our lab. Genomic DNA aliquots will be maintained in long-term storage at HIMB. DNA sequence variation will be summarized with standard diversity indices and with an analysis of molecular variance (AMOVA) using ARLEQUIN vers. 3 (Excoffier et al. 2005).

Coalescence approaches will be used to infer population histories, including growth rates, effective population size and age of founding populations. Phylogenetic methods will include neighbor joining and maximum likelihood algorithms in PAUP version 4.0 (Swofford 2000). Population separations will be defined with using  $\Phi_{ST}$  values and the maximum likelihood approach of MIGRATE vers. 1.7.3 (Beerli and Felsenstein 2001). The key innovation in MIGRATE (relative to conventional  $Nm$  estimates) is that it

estimates asymmetric migration: cases where one region is a source and another is a recipient. The utility of this information for resolving dispersal pathways is readily apparent.

Genotyping of nuclear (microsatellite) markers will be used for reef corals. Development of these markers will follow standard techniques (Toonen 1997; Glenn and Schable 2005). Corals often harbor algal symbionts (zooxanthellae). We will follow a protocol developed at the University of Hawaii to obtain clean coral DNA (unpubl. data). After testing for coral-host specificity (Shearer et al. 2002), samples will be genotyped on an ABI 3100 automated sequencer. Data will be analyzed for standard genetic diversity indices using ARLEQUIN. Assignment tests implemented in the programs BAYESASS and STRUCTURE will be employed to detect the number of populations and patterns of gene flow, including its directionality. This approach was successful in describing genetic diversity and gene flow in a Caribbean coral (Baums et al. 2005a,b).

Population connectivity across the eastern and central Pacific in relation to the NWHI is an essential foundation for prudent management and the potential establishment of no-take zones.

## 11. Funding Sources

The budget below includes our work and that of other related projects using other personnel

### **Budget for 2006 HIMB – NWHI CRER Cooperative agreement**

*Biodiversity Characterization of NWHI in terms of spatial connectivity*

A full copy of the agreement is attached to the other HIMB permit applications (Karl, Bowen, Gates, Rappe)

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1991	B.Sc.	Honours Zoology. Department of Zoology, University of Alberta, Edmonton, AB. Thesis Title: <u>Limitations of laboratory assessments of coelenterate predation: Container effects on the feeding preference of the Limnomedusa, <i>Proboscoidactyla flavicirrata</i></u>

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Society for the Study of Evolution
American Society of Naturalists
Western Society of Naturalists
Society for Integrative and Comparative Biology
American Microscopical Society, Invertebrate Zoology Division

## INVITED REVIEWS:

- Selkoe, K.A. & R.J. Toonen. 2006, *online early*. Microsatellites for Ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters* 6:xxx-xxx.
- Toonen, R.J., 2005. Foundations of gregariousness in barnacles. *Journal of Experimental Biology* 208:1773-1774.

## JOURNAL PUBLICATIONS:

- Selkoe, K.A., B.S. Halpern & R.J. Toonen. *In prep*. Evaluating and ranking the vulnerability of regions within the Northwest Hawaiian Islands Coral Reef Ecosystem Reserve to anthropogenic threats.
- Concepcion, G. S. Kahng, M. Crepeau, H. Benayahu, S. Coles & R.J. Toonen. *In prep*. Origin and phylogeography of an invasive octocoral in Hawaii.
- Toonen, R.J. & A.J. Tyre. *Submission planned in near future*. If marine invertebrates were smart: a simple model for optimal settlement choices of competent larvae. (Manuscript available on request).
- Toonen, R.J. & R.K. Grosberg. *Submission planned in near future*. The importance of testing novel primers: isolation and non-Mendelian inheritance patterns of microsatellite markers from the intertidal crab *Petrolisthes cinctipes*. (Manuscript available on request).
- Diehl, J.M., R.J. Toonen & L. Botsford. *Submission planned in near future*. Interannual variability in the spatial pattern of recruitment of the sand crab, *Emerita analoga*, in California. *Marine Ecology Progress Series*. (Manuscript available on request).
- Toonen, R.J. & R.K. Grosberg. *resubmission planned in near future*. Effects of coastal upwelling on genetic structure in an intertidal crab with planktonic larvae. *Evolution*. (Manuscript available on request).
- Concepcion, G., M. Medina & R.J. Toonen. *In press*. Novel mtDNA intron primers from scleractinian corals. *Molecular Ecology Notes*.
- Daly-Engel, T.S., R.D. Grubbs, K. Holland, R.J. Toonen & B.W. Bowen. *In press*. Multiple paternity assessment in three congeneric species of carcharhinid sharks in Hawaii. *Environmental Biology of Fishes*.
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## INVITED BOOK CHAPTERS:

- Hickman, C.P. Jr., L.S. Roberts, A. Larson, H. I'Anson, et al. 2005. Integrated Principles of Zoology, 13<sup>th</sup> Edition, revised chapters: 14 (Acoelomate Bilateral Animals), 15 (Pseudocoelomate Animals), 16 (Molluscs), 21 (Smaller Protostome Phyla) & 22 (Echinoderms and Hemichordates). McGraw Hill Higher Education, Dubuque, IA.
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## OTHER PUBLICATIONS:

- Toonen, R.J. & R.K. Grosberg 2003. Molecular Genetic Analysis of Recruitment Patterns in the Dungeness Crab, *Cancer magister*. California Sea Grant College Program. Research Completion Reports - [http://repositories.cdlib.org/csgc/rcr/Fisheries03\\_02](http://repositories.cdlib.org/csgc/rcr/Fisheries03_02)
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## RESEARCH GRANTS:

### **Bold = current funding**

Cumulative total funds:		\$3,802,490
2006	\$41,051 rejected	Ecological impact of the invasive coral <i>Carijoa riisei</i> on the deep reef in Hawaii and implications for the NWHI conservation. HISC Research and Technology Grant Proposal (R.J. Toonen, R. Grigg & S. Kahng).
2006	\$863,977 pending	Evolution of population connectivity in sea stars (Rob Toonen, Rick Grosberg, Mike Hart & Maria Byrne). National Science Foundation, Biological Oceanography
2006	<b>\$49,790 awarded</b>	Genetic stock resolution in West Pacific Fisheries: Ehu ( <i>Etelis carbunculus</i> ) and Onaga ( <i>Etelis coruscans</i> ). Western Pacific Regional Fishery Management Council & National Marine Fisheries Service (B. Bowen & R. Toonen).
2005	<b>\$66,082 awarded</b>	Phase one study of Stock Structure in the deepwater snapper Opakapaka ( <i>Pristipomoides filamentosus</i> ) in Hawaii. Western Pacific Regional Fishery Management Council & Hawaii Department of Aquatic Resources (Brian Bowen & Rob Toonen).
2005	\$2,497,192 pending	Integrated Ecosystem Science in American Samoa. NOAA, NOS (Rusty Brainard & Rob Toonen et al.).
2005	<b>\$12,500 awarded</b>	Analysis of genotypes of <i>Acropora</i> spp. corals in the Caribbean. US Geological Survey (R. Toonen & I. Baums).
2005	<b>\$416,891 awarded</b>	Population connectivity of coastal fishery species across the California-Mexico international border, Coastal Environmental Quality Initiative Program (Steve Gaines, Rick Grosberg, Giacomo Bernardi, Rob Toonen, Kim Selkoe, Crow White & Gregorio Benavides).
2005	<b>\$40,534 awarded</b>	Ecology and management implications of an invasive soft coral species, <i>Carijoa riisei</i> in Hawaii, HISC Research and Technology Grant Proposal (R.J. Toonen & S. Kahng).
2005	<b>\$52,018 awarded</b>	Ecology of a specialized nudibranch predator ( <i>Phyllodesmium poindimeii</i> ) and implications for potential biocontrol of an invasive octocoral ( <i>Carijoa riisei</i> ) in Hawaii, HISC Research

		and Technology Grant Proposal (R.J. Toonen & S. Kahng).
2005	\$118,581 rejected	Aquaculture of deep-water exotics for the ornamental trade. Center for Tropical and Subtropical Aquaculture (R. Toonen).
2005	<b>\$1,487,485 awarded</b>	Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve – Hawaii Institute of Marine Biology research partnership. NOAA, NOS, Marine Sanctuary Program (J. Leong, B. Bowen, R. Toonen, et al.)
2005	\$2,500,000 rejected	Application of Genetics to Problems in Conservation. NSF Partnerships in International Research & Education (PIRE), National Science Foundation (F. Allendorf, B. Bowen, R. Toonen, et al.).
2005	\$536,308 rejected	Effects of Genetic Relatedness on Recruitment, Colony Formation and Social Interactions in Tropical Reef Fishes. Animal Behavior, National Science Foundation (R. Toonen, B. Bowen & G. Losey).
2005	\$8,850 awarded	Phylogeny of the coral genus <i>Pocillopora</i> . Grant MacEwan Faculty Improvement award, Province of Alberta (awarded to R.W. Shaw & R.J. Toonen)
2004	<b>\$510, 089 awarded</b>	Phylogeography of Indo-Pacific reef fishes. National Science Foundation, Biological Oceanography (awarded to B. Bowen, S. Karl, R. Toonen, et al.)
2004	\$15,000 awarded	Building basic bioinformatic capacity for the Hawaii Institute of Marine Biology. EPSCoR Hawaii (awarded to R.J. Toonen, J. Leong, M. Rappe, R. Gates & B. Bowen).
2004	\$60,076 rejected	Ecology and management implications of an invasive soft coral species, <i>Carijoa riisei</i> in Hawaii. HISC Research and Technology Grant Proposal (R.J. Toonen & S. Kahng).
2004	\$42,253 rejected	Deep water ecological research on an invasive soft coral species ( <i>Carijoa riisei</i> ) in Hawaii. NOAA Aquatic Invasive Species Research and Outreach Program (R.J. Toonen, S. Kahng & D. Pence, preproposal accepted, full proposal rejected).
2004	\$74,916 rejected	Ecology of a specialized invertebrate predator ( <i>Phyllodesmium poindimiei</i> ) and implications for potential biocontrol of an invasive soft coral species ( <i>Carijoa riisei</i> ) in Hawaii. NOAA Invasive Species Research and Outreach Program (R.J. Toonen, & S. Kahng, preproposal accepted, full proposal rejected).
2004	\$512,827 rejected	Collaborative Research: Maintenance of parapatric boundaries depends on density-dependent dispersal. DEB Ecological Biology Cluster, National Science Foundation (A.J. Tyre, R.J. Toonen, M. Bull, B. Tenhumberg & R. Seppelt, submitted).
2004	<b>\$88,540 awarded</b>	Stock structure in Hawaiian opihi (Gastropoda: <i>Cellana</i> spp.). Hawaii Sea Grant. (Awarded to R.J. Toonen, B. Bowen & B. Holland).
2004	<b>\$87,000 awarded</b>	Genetic stock resolution in deep water snappers Ehu ( <i>Etelis carbunculus</i> ) and Onaga ( <i>Etelis coruscans</i> ). Hawaii Sea Grant. (Awarded to B. Bowen & R.J. Toonen).
2004	\$82,569 rejected	Impact of an invasive invertebrate alien species ( <i>Carijoa riisei</i> ) on coral reef ecology in Hawaii. Hawaii Sea Grant. (R. Grigg, S. Kahng & R.J. Toonen, preproposal accepted, full proposal rejected).
2004	<b>\$14,685</b>	Isolation of Monk Seal microsatellite loci. NOAA National Ocean

	<b>awarded</b>	Service, Marine Mammal Commission. (R. Toonen, B. Bowen & J. Schultz).
2004	\$228,950 rejected	Zoogeography, dispersal history, and impact of a highly invasive marine invertebrate on Pacific deep-water coral communities. NOAA Office of Ocean Exploration. (R. Grigg, S. Kahng, R. Toonen & F. Parrish).
2004	\$1,050,000 rejected	HIMB/OI Joint Research Program Outline: Coral Reef Restoration - Enhanced Coral Recruitment and Survival. Harold K.L. Castle Foundation. (R. Toonen, B. Bowen, M. Rappe, P. Jokiel, R. Gates, J. Leong, D. Ziemann, C. Laidley, A. Friedlander, & G. Pruder).
2004	\$522,537 rejected	Effects of Genetic Relatedness on Recruitment, Colony Formation and Social Interactions in Tropical Reef Fishes. Animal Behavior, National Science Foundation (R. Toonen, B. Bowen & G. Losey).
2004	\$444,306 rejected	Acquisition of high throughput instrumentation for a core marine molecular laboratory. National Science Foundation Major Research Instrumentation grant. (J. Leong, B. Bowen, R. Gates, M. Rappé, R. Toonen).
2004	<b>\$71,910 awarded</b>	Reproduction and developmental characteristics of an alien soft coral ( <i>Carijoa riisei</i> ) in Hawaii. Hawaii Coral Reef Initiative. (Awarded to R. Toonen, Y. Benayahu & S. Kahng).
2004	<b>\$74,933 awarded</b>	The ecology and ecological impact of a highly invasive marine invertebrate in Hawaii's coral reef communities. NOAA Undersea Research Program HURL & Coral Reef Conservation Program. (Awarded to R. Grigg, S. Kahng & R. Toonen).
2003	\$1,039,189 rejected	Collaborative Research: Relationship Between Individual Abundance and Genetic Variation for the Purpose of Designing Marine Protected Areas. Biological Oceanography & Population Biology, National Science Foundation. (R. Toonen, S. Gaines & R. Sagarin)
2003	\$47,408 awarded	The ecology and ecological impact of a highly invasive marine invertebrate in Hawaii's coral reef communities. NOAA Undersea Research Program HURL & National Institute of Undersea Technology. (Awarded to R. Grigg, S. Kahng & R. Toonen).
2003	<b>\$534,000 awarded</b>	Extrinsic and intrinsic factors affecting the resilience of corals to climate change, and their use in designing marine reserves. USGS Biological Resources Division. (Awarded to C. Birkeland, A. Baker, V. Garrison, R. Gates, C. Kellogg, G. Piniak, M. Rappé, R. Toonen, J. Stillman, & R. van Woesik).
2003	\$9,992 awarded	Genetic partitions and stock structure in Hawaiian opihi (Gastropoda: <i>Cellana</i> spp.). Hawaii Sea Grant, Program Development Award, HIMB. (Awarded to B. Bowen, B. Holland, R.J. Toonen & C. Bird).
2003	\$9,986 awarded	Phylogeography and origin of a highly invasive alien octocoral. Hawaii Sea Grant, Program Development Award, HIMB. (Awarded to R.J. Toonen, R. Grigg & S. Coles).
2001	\$330,000 awarded	Genetic Analysis of Recruitment and Dispersal in Coastal Marine Ecosystems. Andrew W. Mellon Foundation, University of California, Davis, CA. (Awarded to R.K. Grosberg).

1999	\$113,797 awarded	Molecular Genetic Analyses of Recruitment Patterns in the Dungeness Crab, <i>Cancer magister</i> . California Sea Grant, University of California, Davis, CA. (Awarded to R.J. Toonen & R.K. Grosberg).
1999	\$8,500 awarded	Conservation of Genetic Diversity on Caribbean Reefs. University Research Expedition Program Grant, University of California, Davis, CA. (Awarded to D.B. Carlon & R.J. Toonen).
1997	\$30,000 awarded	Sir James Loughheed Award of Distinction, Predoctoral Fellowship, Government of Alberta. (Awarded to R.J. Toonen).
1993	\$52,500 awarded	NSERC Post-graduate Scholarship 'B', Natural Sciences and Engineering Research Council of Canada. (Awarded to R.J. Toonen).

### SELECTED INVITED SEMINARS:

2005	3rd International Symposium on Deep-sea corals, Rosenstiel School of Marine and Atmospheric Science, Miami, FL
2005	10 <sup>th</sup> International Coral Reef Symposium Invitation for Evolution of Coral Reef Ecosystems mini-symposium (declined), Okinawa, Japan.
2004	Ecology, Evolution & Conservation Biology, University of Hawaii, Manoa, HI.
2004	National Marine Fisheries Service and Marine Molecular Biotechnology Laboratory, University of Washington, Seattle, WA joint seminar.
2003	School of Natural Resource Sciences, University of Nebraska-Lincoln, NB.
2003	Moss Landing Marine Laboratories, California State University, Moss Landing, CA.
2002	Bodega Marine Laboratory, University of California, Bodega, CA.
2002	Behavioural Ecology Research Group, Simon Fraser University, Vancouver, BC, Canada.
2002	Department of Agronomy & Range Science, University of California, Davis, CA.
2002	Department of Ecology, Evolution & Marine Biology, University of California, Santa Barbara, CA.
2002	Department of Zoology, University of Hawaii, Manoa, HI.
2002	Hawaii Institute of Marine Biology, University of Hawaii, Manoa, HI.
2002	Darling Marine Center, University of Maine, Walpole, ME.
2002	Department of Biology, University of Nebraska, Omaha, NE.
2001	Section of Evolution and Ecology, Center for Population Biology, University of California, Davis, CA.
1999	Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI.

### SELECTED TEACHING EXPERIENCE:

2007	Invited instructor for the joint American Genetics Association, Smithsonian Conservation & Research Center course: Recent Advances in Conservation Genetics; directed by Steve O'Brien, Chief of the Laboratory of Genomic
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- Diversity, National Cancer Institute, USA.
- 2006 Guest lecturer on larval biology in Marine Biology (BIO#301), Department of Zoology, University of Hawaii at Manoa, HI.
- 2005 Guest lecturer on conservation genetics and connectivity in Molecular Phylogenetics (BOT#669), Department of Zoology, University of Hawaii at Manoa, HI.
- 2005 Lecturer on conservation genetics and connectivity in marine systems, Ecology, Evolution and Conservation Biology (EECB) graduate core class (ZOOL#719), University of Hawaii at Manoa, HI.
- 2005 Co-Instructor, Molecular Biology of Corals Workshop, Hawaii Institute of Marine Biology, Coconut Island, Kaneohe, HI.
- 2005 Guest lecturer on connectivity in Corals and Coral Reefs (ZOOL#410), Department of Zoology, University of Hawaii at Manoa, HI.
- 2005 Guest lecturer on introduction to molecular ecology & applications of molecular tools in Benthic Biological Oceanography (OCE628), Department of Oceanography, University of Hawaii at Manoa, HI.
- 2005 Guest lecturer on larval biology in Marine Biology (BIO#301), Department of Zoology, University of Hawaii at Manoa, HI.
- 2005 Advanced topics in Conservation Genetics seminar series (ZOOL#691), Department of Zoology, University of Hawaii at Manoa, HI.
- 2005 Professional development seminar series, Ecology, Evolution and Conservation Biology (EECB), University of Hawaii at Manoa, HI.
- 2004 Co-Instructor, Molecular Biology of Corals Workshop, Hawaii Institute of Marine Biology, Coconut Island, Kaneohe, HI.
- 2004 Guest lecturer on introduction to molecular ecology & applications of molecular tools in Benthic Biological Oceanography (OCE628), Department of Oceanography, University of Hawaii at Manoa, HI.
- 2004 Guest lecturer on larval biology in Marine Biology (BIO#301), Department of Zoology, University of Hawaii at Manoa, HI.
- 2004 Advanced topics in molecular ecology seminar series (ZOOL#691), Department of Zoology, University of Hawaii at Manoa, HI.
- 2003 SOEST graduate student journal club, Guest lecturer: Introduction to coalescent theory. University of Hawaii at Manoa, HI.
- 2003 Co-Instructor, Molecular Biology of Corals Workshop, Hawaii Institute of Marine Biology, Coconut Island, Kaneohe, HI.
- 2001-2002 Guest lecturer on marine ornamentals and the impact of the saltwater aquarium hobby on tropical reefs, Department of Animal Sciences, School of Veterinary Medicine, University of California, Davis, CA.
- 2002 Molecular and Genetic Methods in Ecology IGERT Workshop, Department of Agronomy & Range Science, University of California, Davis, CA.
- 1995-1997 Guest lecturer for Invertebrate Zoology, Section of Evolution and Ecology, University of California, Davis, CA
- 1994-1997 Graduate teaching assistant for introductory biology, animal diversity, and invertebrate zoology, Center for Population Biology, University of California, Davis, CA.
- 1991-1993 Graduate teaching assistant for introductory biology, and invertebrate zoology, Department of Biological Sciences, University of North Carolina, Wilmington, NC.

## SYNERGISTIC ACTIVITIES & PROFESSIONAL SERVICE:

- *Broadening the Participation of Underrepresented Groups in STEM:*

The University of Hawaii is a minority institution, and my research laboratory actively recruits underrepresented minority participants through programs such as the Haumana/MARC Honors Program, SeaGrant MSURF and an NSF funded UMEB project. Minority students supervised to date include (name, origin, home institute):

- Saipologo Toala – Pago Pago, American Samoa Community College (2004)
- Houston Lomae – Majuro, College of the Marshall Islands (2004)
- Megan Lin – Hawaii, UCLA (2004)
- Greg Concepcion – Filipino, University of Maryland (2004-2005)
- Sierra Isalias – Koror, Palua Community College (2005)
- Alexander Alfonso – Pohnpei, College of Micronesia (2005)
- Laurie Sorenson – Hawaii, Hawaii Pacific University (2005)
- Van Nicholas Velasco – Hawaii, UH Manoa (2005-2006)

2002-current	<i>Marine Ecology Progress Series</i> Review Staff Anonymous Referee
1995-current	Reviewed manuscripts for <i>Aquaculture</i> , <i>BioTechniques</i> , <i>Ecology</i> , <i>Evolution</i> , <i>Genome</i> , <i>Journal of Experimental Biology</i> , <i>Journal of Heredity</i> , <i>Journal of Experimental Marine Biology and Ecology</i> , <i>Journal of Plankton Research</i> , <i>Limnology and Oceanography</i> , <i>Marine Biology</i> , <i>Marine Ecology Progress Series</i> , <i>Molecular Ecology</i> , <i>Molecular Phylogenetics and Evolution</i> , <i>Nature</i> , <i>Science</i> , Sea Grant, the National Science Foundation, Natural Environment Research Council (UK), Australian Research Council and several edited book volumes. (currently ~ 25 / yr)
2005	Recognized as an 'Expert of International Standing' by the Australian Research Council (ARC) College of Experts.
2004	Rosenstiel School of Marine and Atmospheric Science outside expert reviewer for annual Smith Prize competition.
1995-1998	Graduate admissions committee, Center for Population Biology, University of California, Davis, CA.
1994-2001	Supervisor for invertebrate care and display aquaria, Section of Evolution and Ecology, University of California, Davis, CA.
1991-1993	Setup and maintenance of invertebrate display aquaria, Department of Biological Sciences, University of North Carolina, Wilmington, NC.
1991-1993	Library acquisitions committee, University of North Carolina, Wilmington, NC.
1991-1992	Graduate advisory and admissions committee, University of North Carolina,

Wilmington, NC.

1991-1992      President of graduate students association, University of North Carolina,  
Wilmington, NC

- *Professional Service*

HIMB marine biology faculty search committee (2006)  
Zoology Department graduate admissions committee (2006)  
Organization and hosting of HCRI reproductive biology of octocorals workshop (2005)  
Organization and hosting of EPSCoR sequence data and phylogenetic analyses workshop (2005)  
Zoology Department entrance exam committee (2005)  
Census of Marine Life planning workshops (2004 - current)  
Northwest Hawaii Islands Scientific Symposium (2004)  
Overview of Research at HIMB – presentation to Honolulu Aquarium Society (2004)  
HIMB Journal discussion group (2004 – current)  
EECB Phylogenetics discussion group (2004 – current)  
Western Pacific Fisheries Management Council coral reef fish stock assessment workshop (2004)  
Chair, Evolutionary & Conservation Genetics faculty search committee (2004)  
Chair, Ecology, Evolution & Conservation Biology graduate committee (2004 – current)  
HIMB Visitors committee (2004 – current)  
Genetic assessment of Hawaiian Cetaceans, WPFC (invited by Paul Dalzell) (2004)  
Palmyra field station consortium planning meeting (2004)  
NWHICRER-HIMB research planning meetings for sanctuary designation (>10 / year)  
National Marine Fisheries Service lobster connectivity workshop (2004)  
Pauley summer course: Molecular Biology of Corals (2003 – current)  
HIMB Parking committee (2003 – current)  
EPSCoR MAP presentation on behalf of Evolutionary Genetics thrust group (2003, 2004)  
Overseeing equipment purchases for EPSCoR evolutionary genetics thrust group (2003 – 2006)  
Overseeing EPSCoR core genetics facility at HIMB (2003 – 2005)

## COLLABORATORS & STUDENT TRAINEES:

- *Scientific Collaborators (Last 4 Years):*

F. Allendorf (UMontana), G. Bernardi (UCSC), R. Grosberg (UC Davis), R. Warner & S. Gaines (UCSB), B. Halpern (NCEAS), D. Zacherl (CS Fullerton), J. Gardiner (Wellington), E. Sotka & S. Palumbi (Hopkins), J. Wares (UGA), B. Stenick (Maine), M. Hart & C. Keever (Simon Fraser, Canada), P. Jensen (UWashington), M. Byrne (USydney, Australia), J. Diehl & L. Botsford (UC Davis), D. Carlon, C. Birkeland, R. Grigg, S. Kahng, R. Gates, M. Rappé, F. Cox, B. Holland & C. Bird (UH Manoa), J. Stillman (Tiburon Center, SFSU), S. Coles & S. Godwin (Bishop Museum), M. Iacchei & K-A. Miller (WEIS, USC), D. Tyre & B. Tenhumberg (UN Lincoln), M. Bull (Flinders, Australia), R. Seppelt (UFZ, Germany), A. Baker (RSMAS), V. Garrison & C. Kellogg (USGS, FL), G. Piniak (USGS, CA) R. van Woesik (James Cook U, Australia) and co-authors above.

- *Graduate / Post-graduate Advisors:*

Joe Pawlik, Martin Posey and Larry Cahoun (UNC Wilmington);  
Rick Grosberg, Mau Stanton, Dennis Hedgecock, Gary Vermeij (UC Davis), and Ron Burton (Scripps)

- *Graduate / Post-doctoral Advisees (N=13) :*

Matt Dunlap (PhD, UH Manoa), Brian Boeing (PhD, UH Manoa), Kimberly Weersing (MSc, UH Manoa), Joe O'Malley (PhD, UH Manoa), Greg Concepcion (PhD, UH Manoa), Matt Iacchei (PhD, UH Manoa), Jon Puritz (PhD, UH Manoa), Michelle Gaither (PhD, UH Manoa), Molly Timmers (MSc, UH Manoa), Derek Skillings (PhD, UH Manoa), Daniel Wagner (MSc, UH Manoa)

Kim Selkoe (post-doc, UH Manoa), Iliana Baums (post-doc, UH Manoa).

- *Graduate Student Committee Member (N=12) :*

Thien Mai (MSc, San Diego State University), Sheri Johnston (PhD, U Maine), Dan Barshis (PhD, UH Manoa), Jennifer Schultz (PhD, UH Manoa), Toby Daley-Engel (PhD, UH Manoa), Kim Andrews (PhD, UH Manoa), Anuschka Faucci (PhD, UH Manoa), Sam Kahng (PhD, UH Manoa), Jeff Eble (MSc, UH Manoa), Dawn Reding (MSc, UH Manoa), Mike Dunford (PhD, UH Manoa), Holly Jessop (MSc, UH Hilo).

## PUBLIC OUTREACH:

- *Newspaper Exposure:*

11 newspaper articles highlighting research conducted in our lab: e.g., Honolulu Star-Bulletin, Sunday July 20, 2003. Headline: "Cutthroat Coral: An invasive species first seen 31 years ago now threatens black coral beds off Maui" pgs A1, A7.

- *Popular Literature Publications:*

Toonen, R.J. 1996-current. More than 100 popular literature articles on aspects of coral reef conservation, invertebrate husbandry, and ornamental culture for *Advanced Aquarist Online Magazine*, *Reefs.org*, *Aquarium Fish Magazine*, *Freshwater and Marine Aquarium (FAMA) Magazine*, *Tropical Fish Hobbyist*, *Marine Fish & Reef USA Annual*, *Koralle (German)*, *Les Lettres Récifales (French)*, *Practical Fishkeeping (UK)*, and *ReefArt (Italian)*, magazines (complete list available online at <http://www2.hawaii.edu/~toonen/publications.html#aquarium>)

- *Selected Invited Presentations to the Public (N=31):*

Marine Aquarium Conference of North America, 2006. Echinoderm biology for reef enthusiasts. Houston, TX.

Western Marine Conference, 2006. Clear as mud: deep sandbed and plenum-based aquarium designs for coral reef aquaria. Sacramento, CA.

Fourth International Days of Saltwater Aquaristic, 2005. An introduction to deep sandbed technology and an experimental comparison against Jaubert-style aquarium systems. Strasbourg, France.

Marine Aquarium Conference of North America, September 2004. An experimental comparison of plenum and deep sandbed designs for reef aquaria. Boston, MA.

International Marine Aquarium Conference, May 2003. The importance of data: a comparison of the nutrient processing capacity of sediments in marine aquaria. Chicago, IL.

Marine Aquarium Conference of North America, 2001. Beyond Fragging Corals: Culturing Coral Reef Invertebrates for the Aquarium Trade. National Aquarium, Baltimore, MD.

Western Marine Conference, 2001a. Planktonic Foods: A Test of Commercial Plankton Replacements for Ornamental Culture. Monterey Bay Aquarium, Monterey, CA.

Western Marine Conference, 2001b. The Next Step: Captive Breeding & Rearing the Larvae of Coral Reef Species. Monterey Bay Aquarium, Monterey Bay, CA.

Aquarium Society of Alberta, 2000. Recent Advances in Techniques for the Aquarium Culture of Coral Reef Invertebrates. Biannual meeting and auction, Edmonton, AB, Canada.

- *Other outreach projects:*

Contributing Editor for *Tropical Fish Hobbyist* and *Advanced Aquarist Online* Magazines  
Curriculum Development for the aquarium program of the Canadian (CAZA) and American Association of Zoos and Aquariums (AAZA) Technology Certification Programs

## REFERENCES:

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Kaneohe, Hawaii  
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## PERSONAL INFORMATION:

*Citizenship:* Canadian

*U.S. immigration status:* Permanent resident (Green card)

